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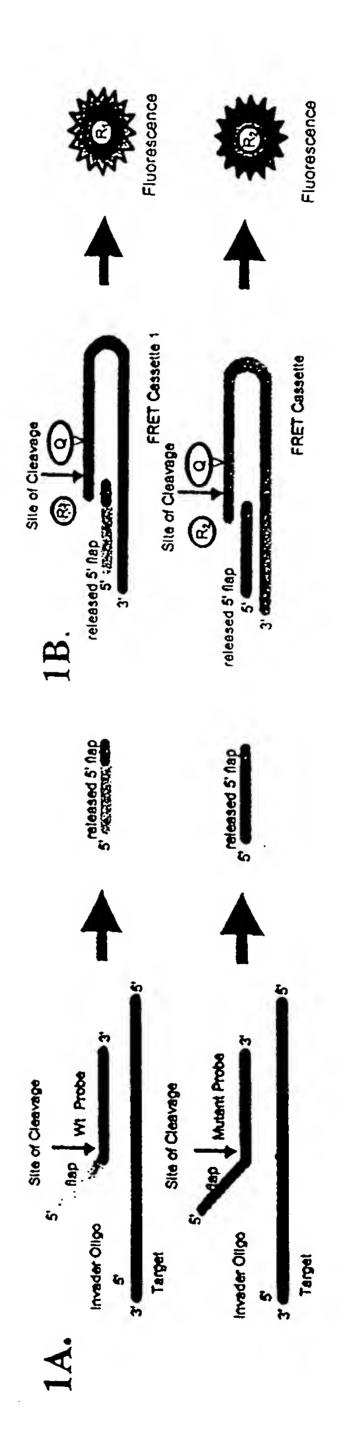
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#### FIGURE 2

## Automated primer selection for multiplex PCR using Invader<sup>TM</sup> Creator Primer Designer v 1.3.3

Multiplex PCR commonly requires extensive optimization to avoid biased amplification of select amplicons and the amplification of spurious products resulting from the formation of primer-dimers. In order to avoid these problems, we have designed *Invader* TM *Creator Primer Designer v1.3.3* software for the automated selection of multiplex primers. Beginning with a set of user defined sequences and corresponding SNP locations, *Invader* TM *Creator Primer Designer* defines an "Invader TM footprint" (the minimal amplicon required for Invader TM detection) for each sequence. Primers are designed outward from the "Invader TM footprint" and evaluated against several criteria, including the potential for primer-dimer formation with previously designed primers in the current multiplexing set. *Invader* TM *Creator Primer Designer* continues through multiple iterations of the same set of sequences until primers against all sequences in the current multiplexing set can be designed.

#### 2A. (SEQ ID NO:263)

#### 2B. (SEQ ID NO:263)

- f,cgggctacccatgggaca,59.38 (SEQ ID NO:333)
  r,tctggtattaagccgtaatttgcatgattga,60 (SEQ ID NO:334)
- Figure 2. Creation of 101 primer sets from sequences available for analysis on the Invader<sup>TM</sup> Medically Associated Panel using Invader TM Creator Primer Designer v 1.3.3. (A) Sample input file of a single entry. Information includes TWT SNP#, short name identifier, and sequence with the SNP location indicated in brackets. (B) Sample output file of the same entry. Information includes the sequence of the "Invader footprint" (capital letters flanking SNP site), forward and reverse primer sequences (bold), and their corresponding Tm's.



#### "Replacement Sheet"

# Figure 5A

#### CYP2D6 PCR amplification:

Primers:

Triplex PCR protocol

Exons 1 & 2 (2036 nt)

2D6L1F1: 5' - CTGGGCTGGGAGCAGCCTC - 3' (SEQ ID NO:335)

2D6L1R1: 5' - CACTCGCTGGCCTGTTTCATGTC - 3' (SEQ ID NO:336)

Exons 3, 4, 5, & 6 (1683 nt)

2D6L2F: 5' - CTGGAATCCGGTGTCGAAGTGG - 3' (SEQ ID NO:337)

2D6L2R2: 5' - CTCGGCCCCTGCACTGTTTC - 3' (SEQ ID NO:338)

Exons 7, 8, & 9 (1754 nt)

2D6L3F: 5' - GAGGCAAGAAGGAGTGTCAGGG - 3' (SEQ ID NO:339)

2D6L3R5B: 5' - AGTCCTGTGGTGAGGTGACGAGG - 3' (SEQ ID NO:340)

Monoplex PCR protocol

CYP2D6 nucleotides 506 – 856 (\*10 & \*21)

forward (1221-09-01): 5' – ggtagtgaggcaggt –3' (SEQ ID NO:341)

reverse (1221-09-02): 5' – gcttctggtaggggag – 3' (SEQ ID NO:342)

CYP2D6 nucleotides 1335 – 1616 (\*11 & \*17)

forward (1221-09-03): 5' – aaataggactaggacctgt –3' (SEQ ID NO:343)

reverse (1221-09-04): 5' – gggtcccacggaaat – 3' (SEQ ID NO:344)

CYP2D6 nucleotides 2092 – 2582 (\*4, \*6 & \*37)

forward (1221-09-05): 5' – catggccacgcg –3' (SEQ ID NO:345)

reverse (1221-09-06): 5' - ccggcacctctcg - 3' (SEQ ID NO:346)

CYP2D6 nucleotides 2977 – 3146 (\*3 & \*33)

forward (1221-09-07): 5' – ccgtcctcctgcat –3' (SEQ ID NO:347)

reverse (1221-09-08): 5' - cactctcaccttctcca - 3' (SEQ ID NO:348)



#### "Replacement Sheet"

# Figure 5B

CYP2D6 nucleotides 3294 - 3494 (\*2 R296C & \*7)

forward (1221-09-09): 5' – gttctgtcccgagtatg –3' (SEQ ID NO:349) reverse (1221-09-10): 5' – tgcactgtttcccaga – 3' (SEQ ID NO:350)

CYP2D6 nucleotides 3589 – 3918 (\*25, \*26 & \*29)

forward (1221-09-11): 5' - ctgacctcctccaacat -3' (SEQ ID NO:351) reverse (1221-09-12): 5' - gggctatcaccaggt - 3' (SEQ ID NO:352)

CYP2D6 nucleotides 4316 – 5226 (\*2, \*27, \*31 & \*32)

forward (1221-09-13): 5' – ctgacctcctccaacat –3' (SEQ ID NO:353) reverse (1221-09-15): 5' – gggctatcaccaggt – 3' (SEQ ID NO:354)



### "Replacement Sheet"

# FIGURE 14

Primer Name	Sequence	Size	Oligo TM	Amplicon Size
primer pair 1	AAG GCT TTG CAG GCT TCA (SEQ ID NO:355)	18 bases	64.3	
primer pair 1	GCT CGG ACT ACG GTC ATC A (SEQ ID NO:356)	19 bases	65.3	1460 bp
primer pair 2	TGG AAT CCG GTG TCG AAG (SEQ ID NO:357)	18 bases	63.4	
primer pair 2	GAA ATC TCT GAC GTG GAT AG (SEQ ID NO:358)	20 bases	58.8	942 bp
primer pair 3	GTA CCT CCT ATC CAC GTC A (SEQ ID NO:359)	19 bases	61.7	
primer pair 3	CAC TCC TTG TTG CCT CCT A (SEQ ID NO:360)	19 bases	62.2	866 bp
primer pair 4	GCA AGA AGG AGT GTC AGG G (SEQ ID NO:361)	19 bases	64.1	
primer pair 4	CTG TGG TGA GGT GAC GAG G (SEQ ID NO:362)	19 bases	66.1	1748 bp

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o He N	Nucleotide position/	Olion Type	<u>\$</u>	Poly-	Assay Olino Sequence
CYP2D6 3259insGT	3259insGT	Target	FAM	INS	TGTCCCCAAAGCGCTCCACCTCATGAATCACGGCAGTGTAGGGCAT (SEO ID NO.411)
CYP2D6 3259insGT	3259insGT	Target	RED	OEL ,	TGTCCCCAAAGCGCTGCACCTCATCACGGCAGTGGTGGTGGCAT (SEQ ID NO.412)
CVP206 FA10K	3853G>A	lovader	INC.		CATCRGTGAAGGATGAGGCCGTCTGGT /SEO ID NO.413\
	3853G>A	Probe	RED	ပ	ACGGACGCGGAGGAGAGCCCTTCCGV (SEO ID NO 414)
	3853G>A	Probe	FAM	V	CGCGCCGAGGAAGAAGCCCTTCCGCV (SEQ ID NO:415)
	3853G>A	Target	RED	၅	GGAAGCGGAAGGGCTTCTCCCAGACGGCCTCATCCTTCAGCACYGATGAC (SEQ ID NO:416)
CYP2D6 E410K	3853G>A	Target	FAM	A	GGAAGCGGAAGGGCTTCTTCCAGACGGCCTCATCCTTCAGCACYGATGAC (SEQ ID NO:417)
20,0	4.0,00		i d		
CYP206 G42K	124G>A	Probe	NONE	ď	GGCCCCCIGCCCI (SEQ ID NO:418)
250	12404	1100G		,	ACCORDO ACCORDO A ACCORDO A ACCORDO AC
CYPZD6 G4ZR	124G>A	Frose	TAM	<b>₹</b> (	CGCGCCGAGGACGC I GGGCAACCV (SEQ ID NO:420)
S GAZK	124GA	Temet	עבט ע	2 <	ACCAGGI I GCCCGGGCAGI GGCAGGGGCCI G (SEQ ID NO.421)
CTP2U0 GAZE	124678	laine.	NA.	<b>T</b>	AGCAGGI I GCCCAGCAGGI GGCAGGGGGCC I G (SECTIO NO. 422)
CVP2DR R201H	1943G>4	Invader	ANCIN		CRECECTECGARTACGACCCTCT (SEC) ID NO 423)
	1943G>A	Probe	FAM	ڻ	CGCGCGAGGCTTCCTCAGGCTGV (SEO ID NO:424)
CYP2D6 R201H	1943G>A	Probe	RFD	A	ACGGACGCGGACTTCCTCAGGCTGCV (SEO ID NO.425)
CYP2D6 R201H	1943G>A	Target	FAM	S	TCCAGCAGCCTGAGGAAGCGAGGGTCGTACTCGAAGCGCCC (SEO ID NO.426)
CYP2D6 R201H	1943G>A	Target	RED	A	TCCAGCAGCCTGAGGAAGTGAGGGTCGTCGTACTCGAAGCGGCGCC (SEQ ID NO 427)
36 R440H	4042G>A	Invader	NONE		CCCTCCCCTCCCCACAGGCCT (SEQ ID NO:428)
CYP2D6 R440H	4042G>A	Probe	FAM	ပ	CGCGCCGAGGGCCGTGCCV (SEQ ID NO:429)
CYP2D6 R440H	4042G>A	Probe *	RED	A	ACGGACGCGGAGACCGTGCCTV (SEQ ID NO:430)
CYP2D6 R440H	4042G>A	Target	FAM	ß	CCCGAGGCATGCACGCGGCGTGTGGGGGGGGGGGGGGC (SEQ ID NO:431)
CYP2D6 R440H	4042G>A	Target	RED	A	GGC (SEQ ID NO
			Į.		
CYPZD6 V11M	31G>A	Invader	NONE L	,	AGAGGGGGCCCCIGGCCI (SEQ ID NO:433)
	31G>A	Probe	FAM	ى د	CGCGCCGAGGGIGATAGTGGCCATCTTCV (SEQ ID NO.434)
CTPZDO VIIM	3107A	-100e	אבת		ACCOMOGRACIONO ACTATO ACCOMO A
CYPZUG V11M	31G>A	Target	FAM	9 <	GCAGGAAGATGGGGGTATGATGGGGGCCAGGGGCTTCTAG (SEQ ID NO:436)
CTP2CO VIIM	A 2016	ader	אבט		SCAGGAAGA I GGCCACIA I CA I GGCCAGGGGCACCAGIGCI I CI AG (SEQ ID NO: 437)
CYP2D6 V338M	3183G>A	Invader	HNCN		GGCCGTGTCCAACAGGATCGACGACT (SEO ID NO-438)
CYP2D6 V338M	3183G>A	Probe	RED	၅	ACGGACGCGGAGGTGATAGGGCCAGGTGV (SEO ID NO.439)
	3183G>A	Probe	FAM	V	CGCGCCGAGGATGATAGGGCAGGTGCV (SEQ ID NO:440)
6 V338M	3183G>A	Target	RED	9	CGCCGCACCTGCCCTATCACGTCGTCGATCTCCTGTTGGACACGGCCTG (SEQ ID NO:441)
CYP2D6 V338M	3183G>A	Target	FAM	A	CGCCGCACCTGCCCTATCATGTCGTCGATCTCCTGTTGGACACGGCCTG (SEQ ID NO:442)
	19G>A	Invader	NONE		TGGCCACTATCAYGGCCAGGGGCAA (SEQ ID NO:443)
CYP2D6 V7M	19G>A	Probe	RED	တ	ACGGACGCGAGCCAGTGCTTCTAGCCV (SEQ ID NO:444)
CYP2D6 V7M	19G>A	Probe	FAM	<b>4</b>	CGCGCCGAGGTCAGTGCTTCTAGCCCV (SEQ ID NO:445)
W \7M	19G>A	Target	RED	ပ	TATGGGGCTAGAAGCACTGGTGCCCCTGGCCRTGATAGTGGCCATC (SEQ ID NO:446)
CYP2D6 V7M	19G>A	Target	FAM	A	TATGGGGCTAGAAGCACTGATGCCCCTGGCCRTGATAGTGGCCATC (SEQ ID NO:447)
			i i		
		Invader	NONE		GCTGGGCTGGGTCAGGTCATCT (SEQ ID NO:448)
		Probe	RED	SNI	ACGGACGCGGAGCTGTGCTCAGTTAGCAGV (SEQ ID NO:449)
		Probe	FAM		CGCGCCGAGGCGTCAGTTAGCAGV (SEQ ID NO:450)
_		Target	REO	INS	ATGAGCTGCTAACTGAGCACAGGATGACCTGGGACCCAGCCC (SEQ ID NO:451)
CYP2D6 2549A>del		Target	FAM	DEL	ATGAGCTGCTAACTGAGCACGGATGACCTGGGACCCAGCCC (SEQ ID NO:452)
1207170401		100,000	Į V		Car Cit
CIPZDO 1/0/12del		Illyader	NONE	9	CAGGCGCCTCCTCGGTCACCT (SECTION NO.453)
		Probe	L AM		CGCGCCGAGGCACTGCTCCAGCGAV (SEQ ID NO:454)
		Frone	KED	ואוני ואוני	
		Target	7 Y Y	S i	AGAMOTOCITO ACCACIONO CONTOCO CONTOCO (SEQUID NO:456)
CIPZUD 1/0/12del					

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SNP Name	change	Oligo Type	Dye morph	morphism Assay Oligo Sequence
CYP2D6 221C>A s		Invader	Æ	CCCACCATCCATGTTTGCTTCTGGTRGGGSAC (SEQ ID NO:506)
CYP2D6 221C>A s		Probe	FAM C	CGCGCCGAGGGCCTCAGCACCTCTGV (SEQ ID NO.507)
CYP2D6 221C>A s		Probe	RED A	ACGGACGCGGAGTCCTCAGGACCTCTGCV (SEQ ID NO:508)
CYP2D6 221C>A s		Target	FAM C	GGCGGCAGAGGTGCTGAGGCTSCCCYACCAGAAGCATGGATGGTGGGTG (SEQ ID NO:509)
CYP2D6 221C>A s		Target	RED A	GGCGCCAGAGGTcCTGAGGATSCCCYACCAGAAGCATGGATGGTGGGTG (SEQ ID NO:510)
CYP2D6 223C>G as		Invader	NONE	GGAGGGCGCCAGAGGTSCTGAGGMTT (SEQ ID NO:511)
CYP2D6 223C>G as		Probe	RED C	ACGGACGCGCGCACCAGAAGCAAacV (SEQ ID NO:512)
CYP2D6 223C>G as		Probe	FAM G	CGCGCCGAGGCCCCACAGAAGCV (SEQ ID NO:513)
CYP2D6 223C>G as		Target	RED C	ATGITTGCTTCTGGTaGGGGAGCCTCAGcACCTCTGCCGCCCTCCAG (SEQ ID NO.514)
CYP2D6 223C>G as		Target	FAM	ATGITTGCTTCTGGTgGGGCAaCCTCAGgACCTCTGCCGCCCTCCAG (SEQ ID NO:515)
CYP2D6 223C>G s		Invader (mutant)	NONE	CCACCCATGITTGCTGGTGGGGGT (SEQ ID NO:516)
CYP2D6 223C>G s	South the state of	Invader (wid-type)	NONE	ACCCACCATCCATGTTTGCTTCTGGTRGGGT (SEQ ID NO.517)
CYP2D6 223C>G s		Probe	RED C	ACGGACGCGGAGGAGCTCAGGACCTCV (SEQ ID NO:518).
CYP2D6 223C>G s		Probe ***	FAM G	CGCGCGAGGCATCAGGACCTCTGV (SEQ ID NO.519)
CYP2D6 223C>G s		Target	RED : C	GGCGGCAGAGGTGCTGAGGCTCCCCGACCAGAGCA
CYP2D6 223C>G s	*	Target	FAM G	GGCGGCAGAGGTcCTGAGGATGCCCcACCAGAAGCATGGATGGTGGGTGA (SEQ ID NO:521)
Copy Number Designs	Gene	Oligo		Sequence
E	2D6	2D6 Invader	CCCGCGCCAC	CCCGCGCCCACCTGAGCC (SEQ ID NO:522)
ш	Alpha Actin	Alpha Actin Invader	AGGAGTAGCC	AGGAGTAGCCACGCTCGGTGAGGATCTTCATT (SEQ ID NO:523)
3	2D6	Probe Arm3 2D6	ACGGACGCGG	ACGGACGCGGAGTTACAGCACAGGTGC (SEQ ID NO:524)
В	Alpha Actin	Probe Arm1 Alpha Actin	cececceaee	CGCGCCGAGGCAGGTAGTCGGTGAGATC (SEQ ID NO:525)
យ	2D6	Synthetic Target Alpha Act	GGACCGCACC	Synthetic Target Alpha Aci GGACCGCACCTGTGCTGTAaGCTCAGTGTGGCGCGCGGGGC (SEQ ID NO:528)
<b>B</b>	Alpha Actin	Synthetic Target 2D6	CGCGATCTCA	CGCGATCTCACCGACTACCTGAATGAAGATCCTCACCGAGCGTGGCTACTCCTTC (SEQ ID NO:527)

FRET SEQUENCES SEQ ID 242 (FRET probe FAM): Y-tct-X-agc-cgg-ttt-tcc-ggc-tga-gac-ctc-ggc-gcg-hex

SEQ ID 243 (FRET probe RED): Y-tct-X-agc-cgg-ttt-tcc-ggc-tga-gac-tcc-gcg-tcc-gt-hex